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FRONTIERS IN LIFE SCIENCE

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GolgiSeeing™ <Golgi Apparatus Green>

Catalog NO. FDV-0053

Product Background

The Golgi apparatus is an organelle that plays various roles in physiological functions and is known as a central component of the protein secretory pathway. The Golgi apparatus has unique multiple-layered cisternal membrane structures, with subdivided structures such as cis-Golgi, which is responsible for reciprocal vesicular transport with the endoplasmic reticulum (ER), and trans-Golgi, which is the starting point of the secretory pathway. Since dynamic morphological changes of the Golgi apparatus are essential for secretory function and dysfunction of the Golgi apparatus has been implicated in a number of diseases, it is expected that the Golgi apparatus will be observed by live cell imaging.

Two major methods have been used to stain the Golgi apparatus in living cells: The first is fluorescent staining using fluorescently labeled ceramide derivatives (hereafter ceramide-FL). Ceramide lipids accumulate in the Golgi apparatus during the metabolic pathway, and ceramide-FLs have been used to visualize the Golgi apparatus. However, 1) The Golgi selectivity of ceramide derivatives is low, 2) Ceramide derivatives also localize to the ER, etc., and 3) Ceramide derivatives have high cytotoxicity and are quickly metabolized intracellularly. The second staining method is to overexpress fluorescent proteins by fusing them to Golgi-specific expressed proteins (such as Giantin, *N*-acetylgalactosaminyltransferase, etc.). While this method allows visualization of the Golgi apparatus with a high degree of specificity, it requires prior plasmid transfection, making it impossible to immediately observe the Golgi apparatus when necessary. Furthermore, there are concerns overexpression of specific marker genes may affect physiological functions of the Golgi apparatus. **GolgiSeeing™** is a novel small molecule fluorescent reagent that utilizes a Golgi apparatus selective localization motif discovered by Dr. Shinya Tsukiji's group at Nagoya Institute of Technology. Unlike conventional ceramide-FLs, it can stain the Golgi apparatus with a simple protocol that requires only 10 minutes of addition to the culture medium. It also shows higher Golgi specificity than ceramide-FLs, allowing Golgi apparatus-focused analysis (**Figure 1**). The GolgiSeeing™ can visualize the Golgi apparatus of target cells at any desired timing without genetic manipulation and without bias to physiological functions caused by overexpression, allowing the dynamic behavior of the Golgi apparatus to be observed under more physiological conditions. Under the non-wash protocol, GolgiSeeing™ visualizes not only the Golgi apparatus but also the plasma membrane. This property allows simultaneous imaging of the Golgi apparatus and cell morphology (judged by plasma membrane).

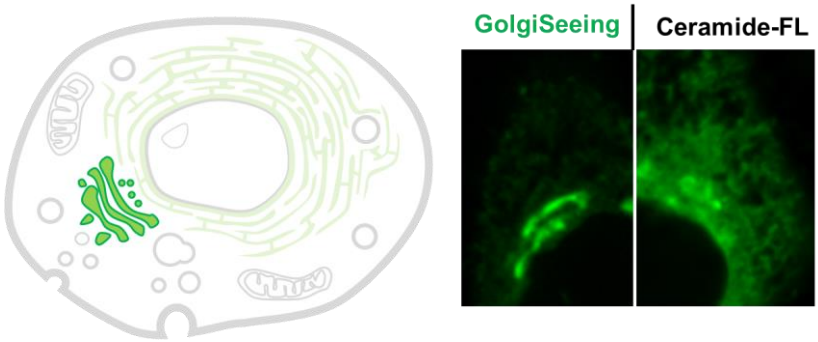


Figure 1. Overview image of GolgiSeeing™

GolgiSeeing™ (original compound name; **mgc^{3Me}FDA** in Ref.1) has a unique structure consisting of fluorescein diacetate (FDA) and *N*-myristoylated Gly-Cys dipeptide (mgc) linked by a linker, and three amide bonds in the mgc peptide chain are all methylated (**Figure 2** left, upper). GolgiSeeing™ functions as a Golgi apparatus-selective fluorescent probe through several processes in the cell. FDA is a modified fluorescein quenched to improve cell membrane permeability, and GolgiSeeing™ emits slight fluorescence before use. After GolgiSeeing™ penetrates the cell membrane and enters the cell, acetyl groups are removed by endogenous esterases, and the green fluorescence of fluorescein is restored (**Figure 2** left, middle). The deacetylated form of GolgiSeeing™ transiently localizes mainly to the ER and Golgi apparatus by the effect of *N*-myristoyl groups and is *S*-palmitoylation by endogenous *S*-palmitoylation enzymes. The Golgi apparatus selective localization motif is formed by adding palmitic acid to the Cys side chain by enzymatic action (**Figure 2** left, lower). Although the intrinsic palmitoylation modification is known to localize to the Golgi apparatus and the plasma membrane, the effect of the three methyl groups biases the equilibrium toward the Golgi, which allows GolgiSeeing™ to selectively visualize the Golgi apparatus (**Figure 2** right).

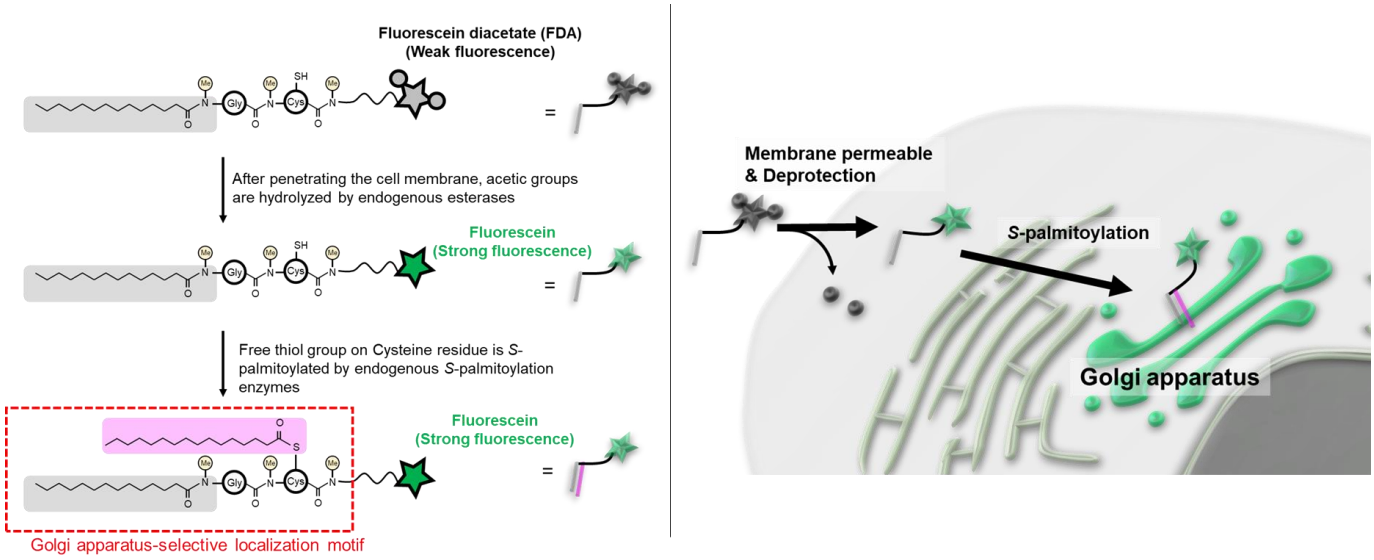


Figure 2. Principle of GolgiSeeing™

Table Live cell Golgi apparatus imaging methods

Category	Genetically encoded	Fluorescent probes	
Staining	Fluorescent protein-fused Golgi apparatus marker proteins	Fluorescent-labeled ceramide derivatives (Ceramide-FL)	**GolgiSeeing™**
Golgi apparatus specificity	High	Low (High ER background)	High (Low ER background)
Protocol	Easy (Exogenous gene transfection)	Complicated (Require stepwise and temperature-controlled protocol)	Easy (Just addition to medium)
Time	> half day (time for protein expression)	>1 hour	10 min
Influence on physiological function	High (Overexpression of exogenous proteins)	High (Exogenous ceramide's toxicity)	Low

Description

Catalog Number: FDV-0053
Size: 0.1 mg
Molecular weight: 1258.5 g/mol
Solubility: Soluble in DMSO
Fluorescent characteristics:
Ex. 440-500 nm (maximum ~480 nm) / Em 500-560 nm (maximum ~520 nm)
Compatible with conventional FITC filter set
*NOTE: GolgiSeeing™ has fluorescein diacetate (FDA), which is quenched by two acetates and emits a slight fluorescence. After hydrolysis of two acetic groups in cells by physiological esterases, fluorescein is exposed and restores strong green fluorescence.

Reconstitution and Storage

Reconstitution: Stock solution recommended concentration 1 mM in 100% DMSO. Add 79 µL of DMSO/vial to prepare 1 mM stock solution.
Storage (powder): Store powder at less than -20ºC
Storage (solution): After reconstitution in DMSO, aliquot and store at less than -20°C.
Avoid repeated freeze-thaw cycles and recommend single use of each aliquot.

Important notice for stability

GolgiSeeing™ has a free-thiol group (See **Figure 2 left**), which is *S*-palmitoylated in cells and essential for Golgi apparatus localization. This thiol group is easily oxidized to form a homodimer in physiological pH buffers, and GolgiSeeing™ -dimer loses its Golgi apparatus localization function and may cause a background signal. GolgiSeeing™ working solution (such as GolgiSeeing™ -containing medium or buffers) should be prepared just before use. Importantly, GolgiSeeing™ **may be gradually oxidized even in DMSO. After reconstitution of GolgiSeeing™ in DMSO, use within one month to maintain good staining results.**

How to use

General procedure for Golgi apparatus-selective staining in live cells

*This procedure is an example of Golgi apparatus-selective staining

1. Prepare 10 µM GolgiSeeing™ in serum-free medium such as DMEM just before use
   NOTE: Empirically optimize and determine the concentration of GolgiSeeing™ for your experiments.
2. Remove the culture medium and wash cells with medium several times
3. Add GolgiSeeing™ -containing medium to cells
   NOTE: Working solution of GolgiSeeing™ prepared in step-1 should be quickly used, as GolgiSeeing™ may be oxidazed to form inactive dimer in medium.
4. Incubate cells at RT for 10 min
   NOTE: Empirically optimize incubation time and temperature for your experiments.
5. Wash cells with **3 mg/ml BSA-containing medium** over two times and add fresh serum-free and phenol red-free medium
   NOTE: GolgiSeeing™ is a highly hydrophobic compound and non-specifically absorbed to plastic or glass surface of imaging chambers. This non-specific absorption may cause the background signal of fluorescent images. **To reduce non-specific signals, we highly recommend using a wash buffer containing BSA** because BSA efficiently binds to excess GolgiSeeing™ and removes it from the surface of imaging chambers. 3 mg/ml BSA-containing basal medium is the preferred choice of GolgiSeeing™ -wash buffer.
6. Observe cells under live cell condition

General procedure for Golgi apparatus and plasma membrane staining in live cells

*This procedure is an example of Golgi apparatus and plasma membrane staining

1. Prepare 2 μM GolgiSeeing™ in serum-free and phenol red-free medium such as DMEM just before use
   NOTE: Empirically optimize and determine the concentration of GolgiSeeing™ for your experiments.
2. Remove the culture medium and wash cells with medium several times
3. Add GolgiSeeing™ -containing medium to cells
   NOTE: Working solution of GolgiSeeing™ prepared in step-1 should be quickly used, as GolgiSeeing™ may be oxidazed to form inactive dimer in medium.
4. Incubate cells at RT for 10 min
   NOTE: Empirically optimize incubation time temperature for your experiments.
6. Observe cells without wash-out step under live cell condition

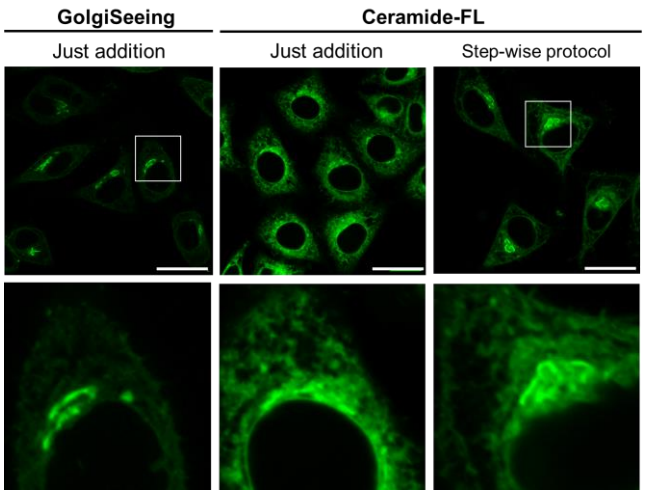
Important Notice of Use

1) GolgiSeeing™ will selectively localize to the Golgi apparatus through the *S*-palmitoylation on the free-thiol group by endogenous *S*-palmitoylation enzymes, as mentioned in **Figure 2**. Note that any drugs inhibiting *S*-palmitoylation may influence the Golgi apparatus-selective staining property of GolgiSeeing™. Especially, alkylation reagents of the free thiol (such as maleimide, iodoacetate, etc.) will critically inhibit *S*-palmitoylation and are incompatible with GolgiSeeing™.
2) GolgiSeeing™ has no fixable functional groups in the molecule and is not fixed by either paraformaldehyde (PFA) or methanol. GolgiSeeing™ is a specialized reagent for live cell imaging applications, not compatible with fixed cell imaging and immunocytochemistry.
3) Long incubation time of GolgiSeeing™ may increase ER-derived fluorescent signal and reduce Golgi/ER ratio. Conduct empirical optimization ranges for your experiments' incubation time and observation time course.

Reference data

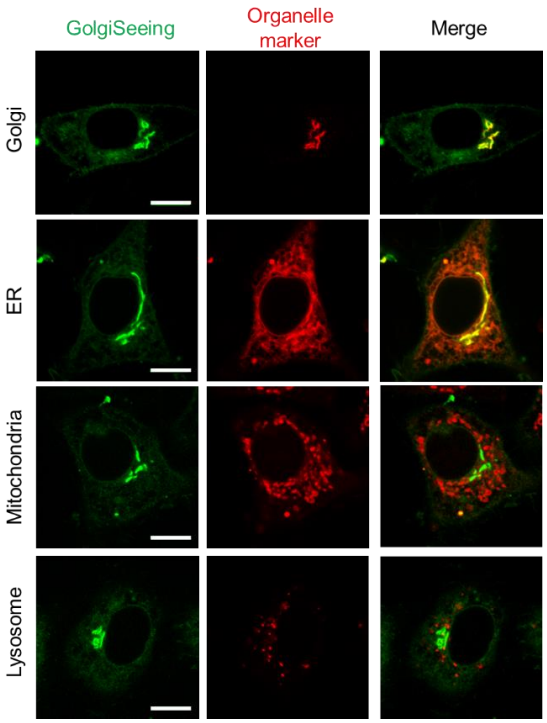
Comparison between GolgiSeeing™ and a ceramide-based reagent

HeLa cells were treated with 10 μM GolgiSeeing™ or 5 μM ceramide-FL (as BSA complex). In the case of GolgiSeeing™, the protocol is a simple addition of GolgiSeeing™ into media final 10 μM and incubated for 10 min. After washing cells with 3 mg/ml BSA containing media, fluorescent images were captured by confocal laser microscopy (Ex 488 nm/Em 500-600 nm). On the other hand, ceramide-FL (BSA complex) -staining was performed by two protocols, simple addition or stepwise temperature-controlled protocol. The later protocol cells were incubated with ceramide-FL (BSA complex) for 30 min at 4°C in HBSS, washed with ice-cold HBSS, and incubated in a fresh culture medium for an additional 30 min at 37°C. Finally, cells were washed with a fresh medium again and observed by confocal laser microscopy. This stepwise protocol requires over 1 hour. The ceramide-FL probe stains not only the Golgi apparatus but also ER structure with high intensity non-specifically. GolgiSeeing™ was able to visualize Golgi apparatus selectively and suppressed non-specific ER staining.



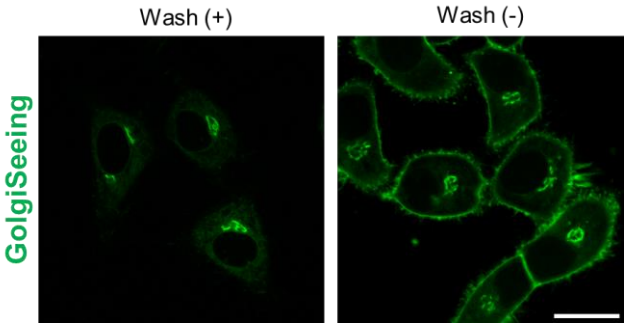
Organelle specificity

HeLa cells were co-stained with GolgiSeeing™ and organelle markers. In the case of the Golgi apparatus marker, overexpression of mCherry-Giantin fusion protein was used. In the cases of ER, mitochondria, and lysosome, each organelle was stained by organelle-specific chemical probes. The fluorescent signal from GolgiSeeing™ is well corresponded with Golgi-marker and weakly overlaps with ER. However, the signals are not matched with mitochondria and lysosomes.



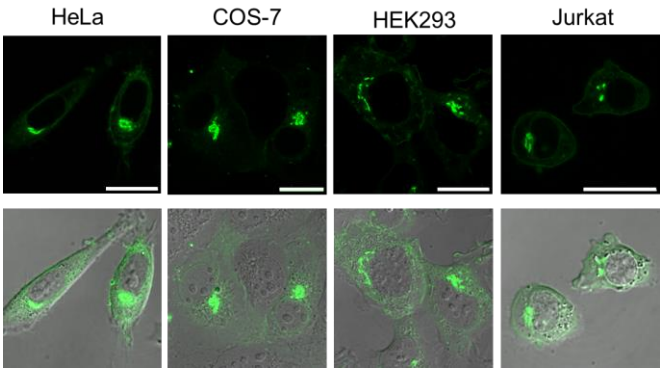
Effect of wash-out step on plasma membrane staining

HeLa cells were treated with 10 μM GolgiSeeing™ for 10 min and observed by confocal laser microscopy (Ex 488 nm/Em 500-600 nm) with or without the wash step. For wash-out, 3 mg/ml BSA-containing medium was used as wash buffer. In the case of non-wash observation, the fluorescent signals of GolgiSeeing™ were observed in not only the Golgi apparatus but also the plasma membrane. As the Golgi apparatus is clearly distinguished from the plasma membrane, non-wash staining is useful for simultaneous observation of the Golgi apparatus and cell morphology (the shape of the plasma membrane). The wash-out step dramatically reduced the fluorescent signal from the plasma membrane.



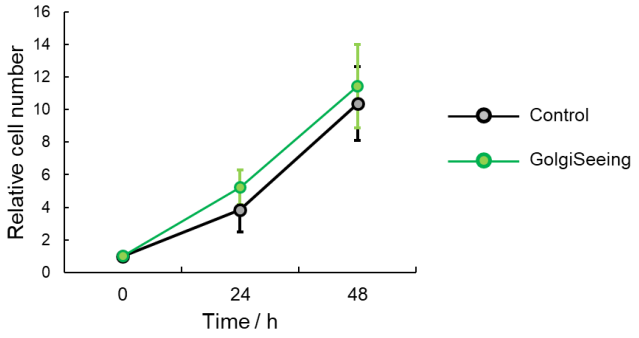
Various cell staining

Four types of cultured cells (HeLa, COS-7, HEK293, and Jurkat) were treated with 10 μM GolgiSeeing™ for 10 min. After cell wash-out, cells were observed by confocal laser microscopy (Ex 488 nm/ Em 500-600 nm). For all cells tested here, GolgiSeeing™ highly selectively stained the Golgi apparatus.



Cellular toxicity

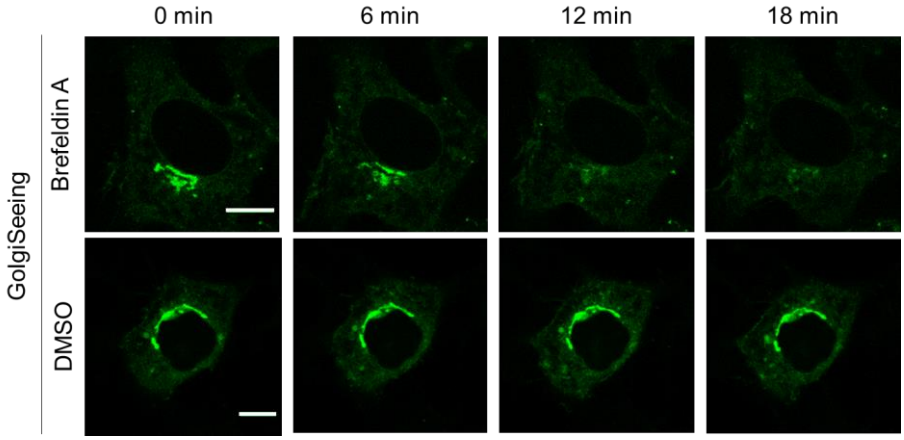
HeLa cells were seeded into a 12-well plate at 0.5 x 10^5 cells/well and cultured for 48 hours with/without 10 µM GolgiSeeing™. After 2, 24, and 48 hours, cell numbers were assessed by a cell counter. GolgiSeeing™ shows little effect on cell proliferation.



Application data

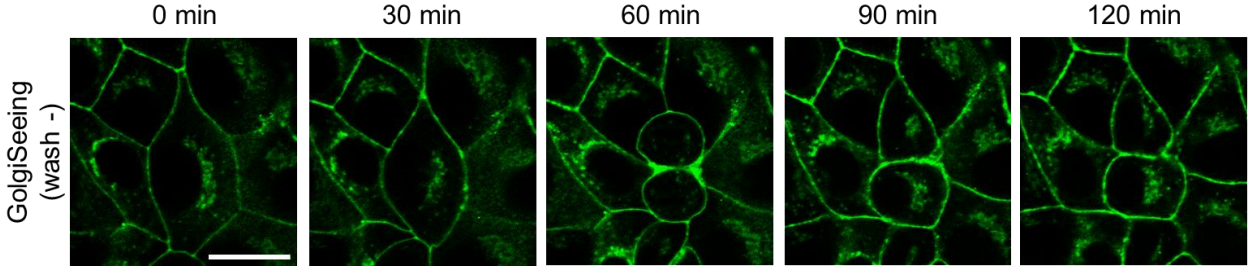
Live cell time-lapse imaging on Brefeldin A-induced collapse of Golgi apparatus

HeLa cells were seeded on glass bottom dishes and treated with 10 µM GolgiSeeing™ for 10 min. After washing cells with 3 mg/ml BSA-containing media, the cells were cultured in brefeldin A (final 1 µM in 0.1% DMSO)-containing medium or 0.1 % DMSO-containing medium as a negative control and observed under live cell condition by confocal microscopy (Ex 488 nm/Em 500-600 nm). In the brefeldin A-treated cells, the fluorescent signal from the Golgi apparatus gradually disappeared.



Live cell time-lapse imaging during cell division

MDCK cells were seeded on a glass bottom dish, incubated with 2.5 µM GolgiSeeing™, and observed for 2 hours by confocal laser microscopy (Ex 488 nm/ Em 500-600 nm) without washing step. As a non-wash procedure, the fluorescent signal of GolgiSeeing™ were observed not only in Golgi apparatus but also in plasma membrane. By imaging of plasma mebrane structures, cell morphology was easily observed. According to the progression of cell division, the Golgi apparatus disappeared (60 min) and was reconstituted in two daughter cells (90-120 min).



Reference

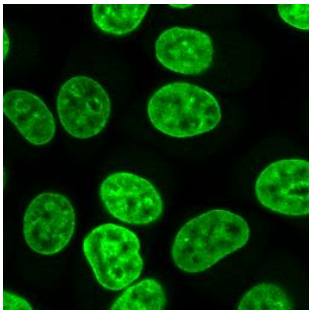
1. Sawada *et al.,ACS Chem. Biol.*, 18, 1047-1053 (2023) Palmitoylation-Dependent Small-Molecule Fluorescent Probes for Live-Cell Golgi Imaging.

Related products

NucleoSeeing™ <Live Nucleus Green>

NucleoSeeing™ is DNA-responsive green dye for monitoring cell nucleus in live cells. As it shows low cytotoxicity and phototoxicity, it is very suitable for long-term live imaging of cell nucleus.

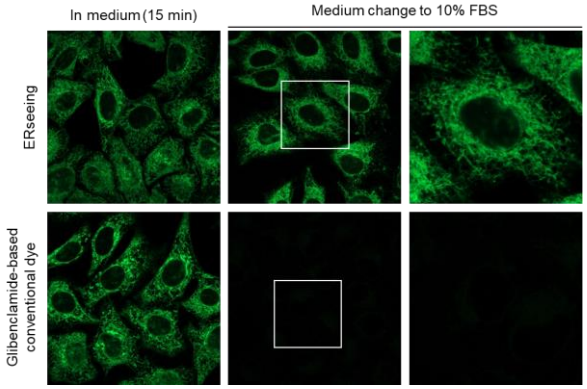
Catalog No. FDV-0029
Size 0.1 mg
Features
- Easy and quick procedure
- Compatible with 10% FBS
- Validated for both adherent cells and floating cells
- Little influence on cellular functions
- Ex/Em: 488 nm/520 nm (commercial FITC filters are available)



ERseeing™ <Endoplasmic reticulum Green>

ERseeing™ is a novel type of ER-staining dye and shows little pharmacological effects compared with conventional glibenclamide-based ER dyes. ERseeing™ is irreversible staining and is compatible with medium change for long-term imaging.

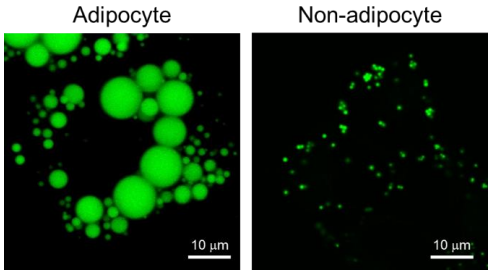
Catalog No. FDV-0038
Size 10 nmol
Features
- Recommended Ex/Em: 509 nm/524 nm
- Lless pharmacological effect on ER proteins
- Suitable for long-term live cell imaging



LipiDye™ II <Lipid Droplet Live Imaging>

LipiDye™ II is a highly sensitive lipid droplet staining dye with extremely photostable property. This dye is the second generation of our previous reagent, LipiDye™. This dye allows us to detect small lipid droplets (<1 μm) in non-adipocytes and to apply into long-term live cell imaging for dynamic lipid droplet movements.

Catalog No. FDV-0027
Size 0.1 mg
Features
- Recommended Ex/Em:400-500 nm / 490-550 nm
- Enable to detect <1 μm lipid droplets
- Suitable for long-term live cell imaging
- Extremely photostable compared with conventional dyes
- Compatible with both live and fixed cells



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